

Microbial Residue and Compounds of Life from Enceladus (MIRACLE)

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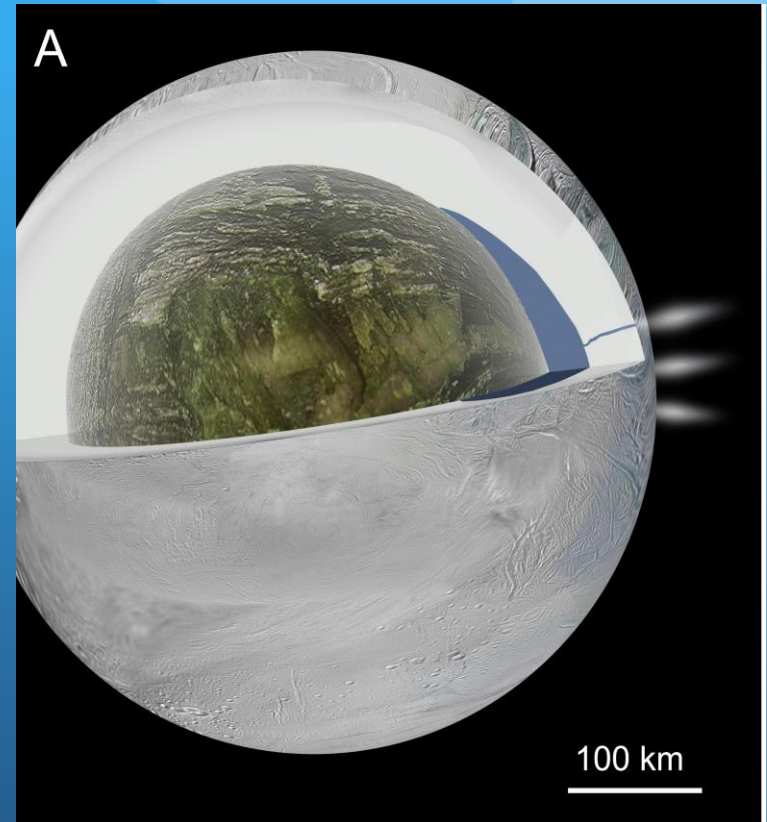
This research was carried out at the California Institute of Technology and the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration.

Outline

- Motivation
- Research Plan
- Experimental design and results Part I
 - Measure concentration & dilution of bacteria into droplets
 - Escape of bacteria into droplets and droplets from bulk fluid
- Summary and Conclusions

Motivation

- Enceladus plumes are known to contain organics—could they contain whole, even living, bacterial cells?
- If cells do exist and manage to find their way into the plumes, how much plume would have to be captured, and at what capture velocity, in order to preserve biosignatures?
- How do we map detected biosignatures to the original source material?

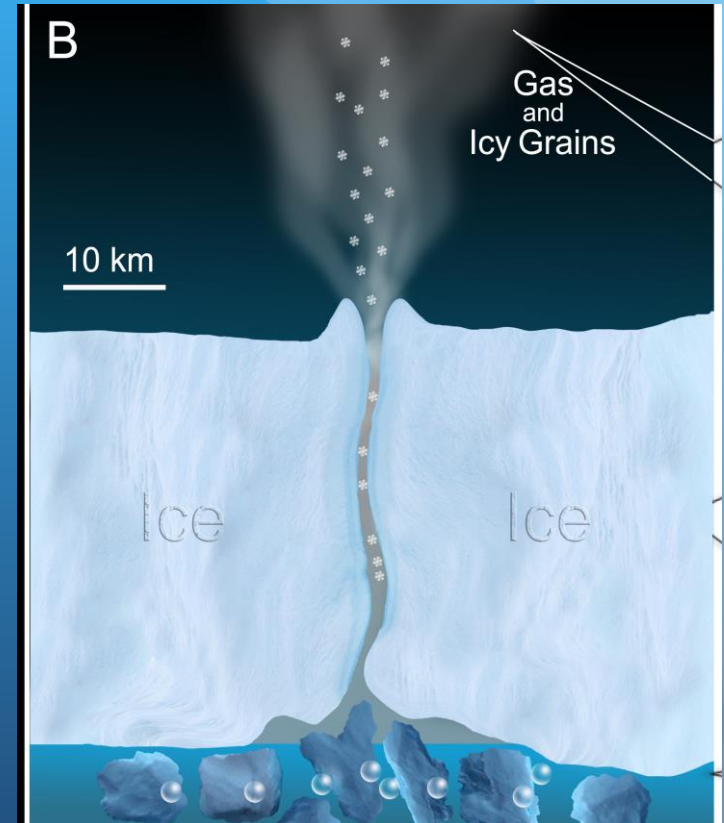


Our goal with this work:

Assuming some biological source material containing known extant life, what would make its way from the bulk fluid into the plumes and what would it look like by the time it is caught and analyzed?

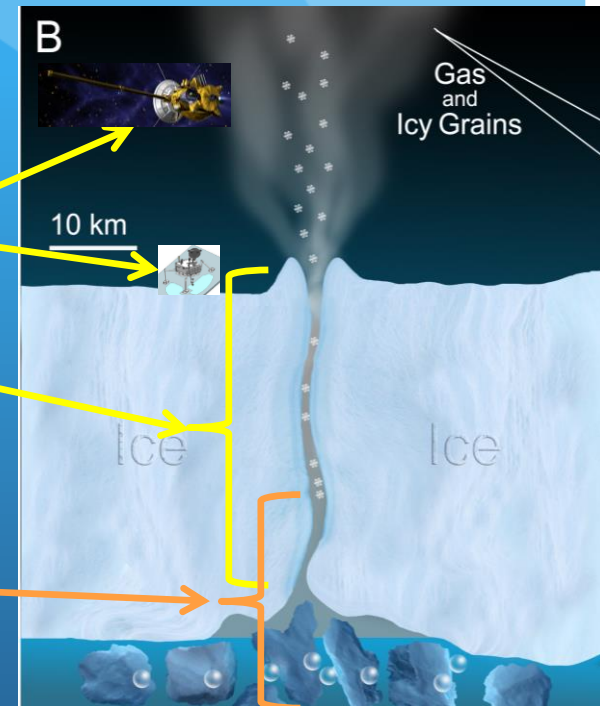
Motivation – how much would we have to catch

- Plume density is low: $(10^{-14} \text{ mL H}_2\text{O})/(\text{mL of plume})$
 - At 10^5 cells/mL (typical Earth ocean value), a 50 km transect could yield 9 cells on a 20 cm x 20 cm collector
- If there are cells in the ocean will they appear in the plumes at the same relative concentration?
 - What would the cells look like after they are caught in various ways?
 - From Enceladus orbit or scooped from surface (tens to hundreds of m/s)
 - From Saturn orbit km/s!

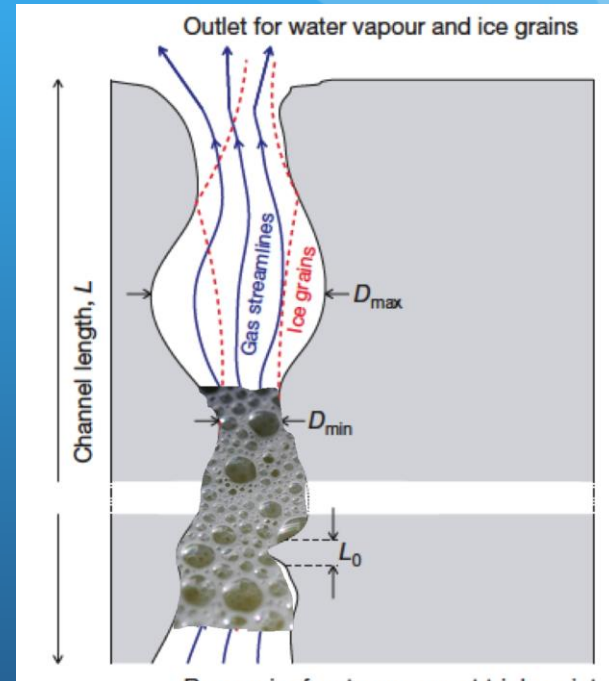
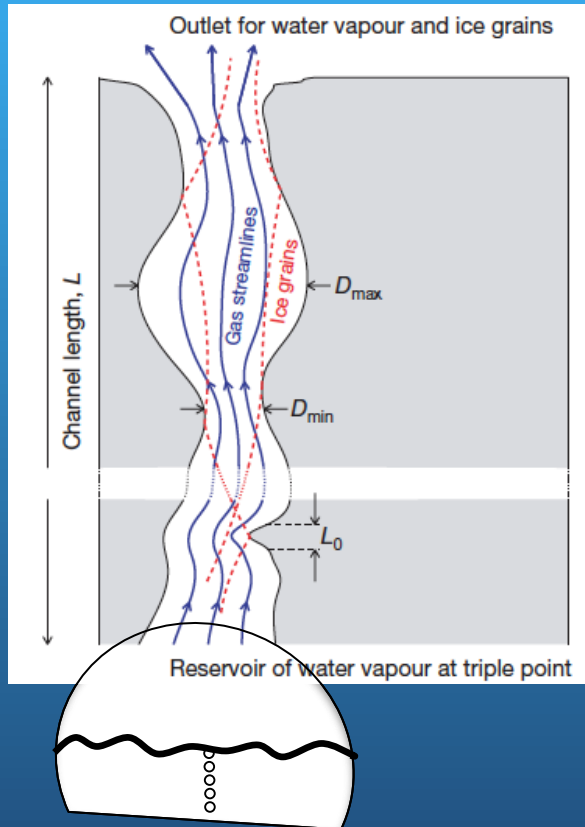


Divide up the problem

- Separate the problem of catching life in the plumes into parts:
 - 3) Someone flies by and catches them or lands and scoops up the snow and inspects
 - 2) Crystals get from their origination point up to ~200 m/s out through the ice into space
 - 1) Life from the ocean gets into flying droplets/ice crystals
- This presentation covers work we're doing on #1 & the low velocity capture approach
- We have separate work in progress on #3 (high velocity capture)
- We're using a few different models for #2 to help bound #1 & #3



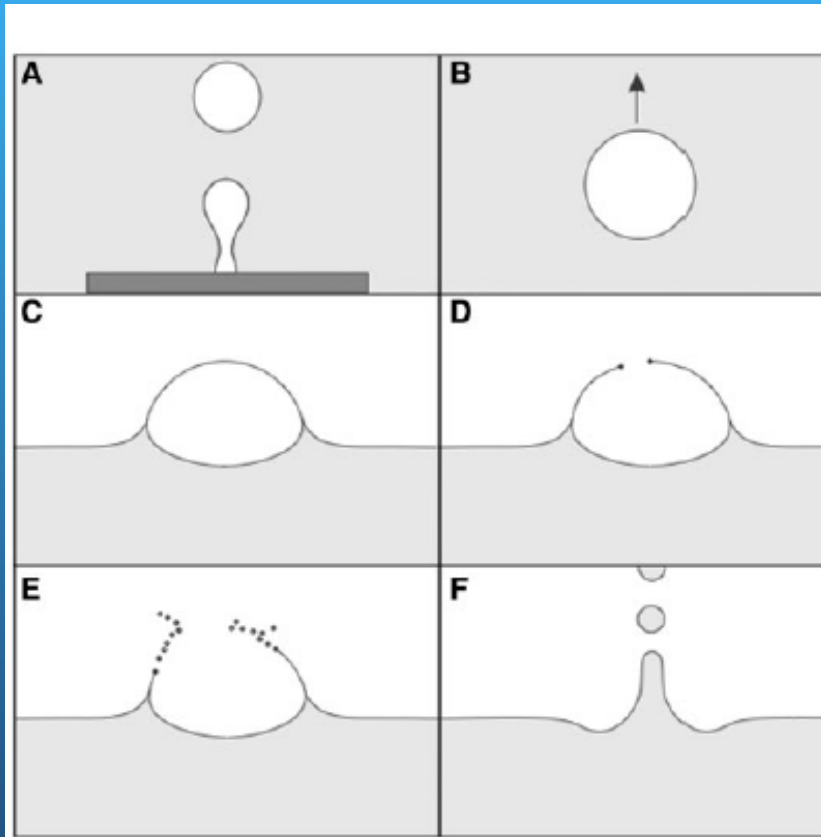
Two Models for Enceladus Plumes



- Reservoir with liquid surface and gas above, venting up column
- Triple point boiling produces film drops and jet drops

- Liquid reservoir with frothy exsolution of CO_2 , froth extends up column
- produces film drops – the bulk liquid surface is buried under the froth

Jet Drops and Film Drops

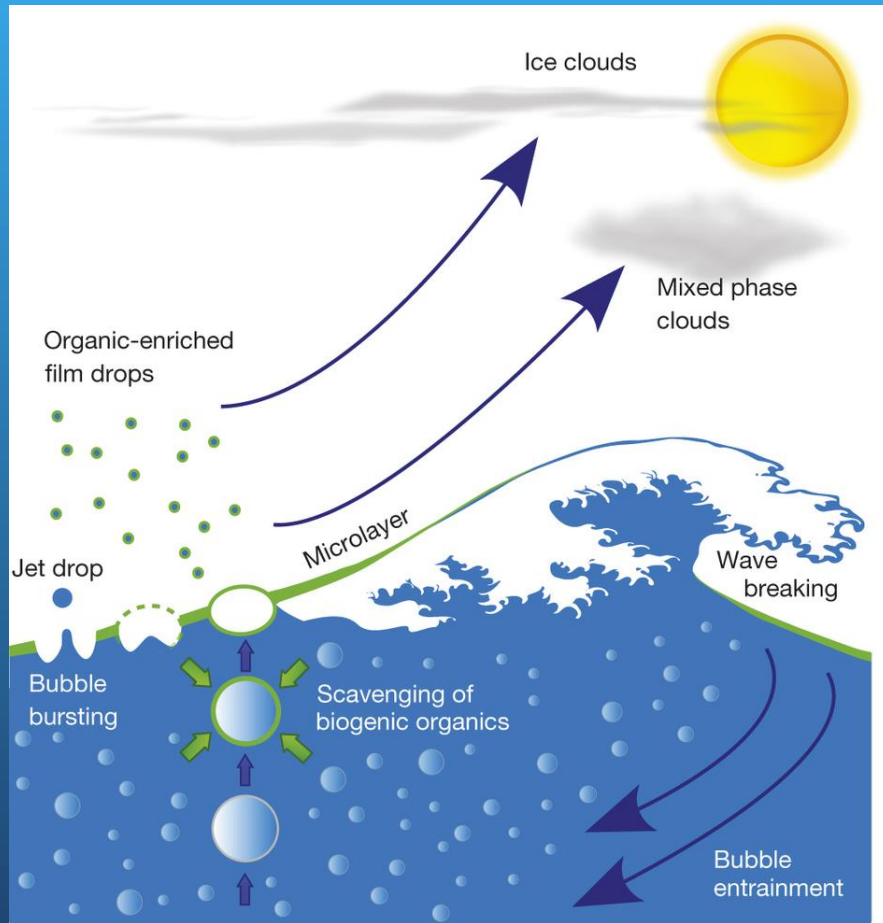


Film Drops

Jet Drops

- Film Drops (left) are formed from the film of a bursting bubble.
- Jet drops (right) are formed when a bubble bursts and fluid rushes in to fill the space occupied by the bubble
- Both types of drop can play a role in dispersing microorganisms that are in the bulk liquid

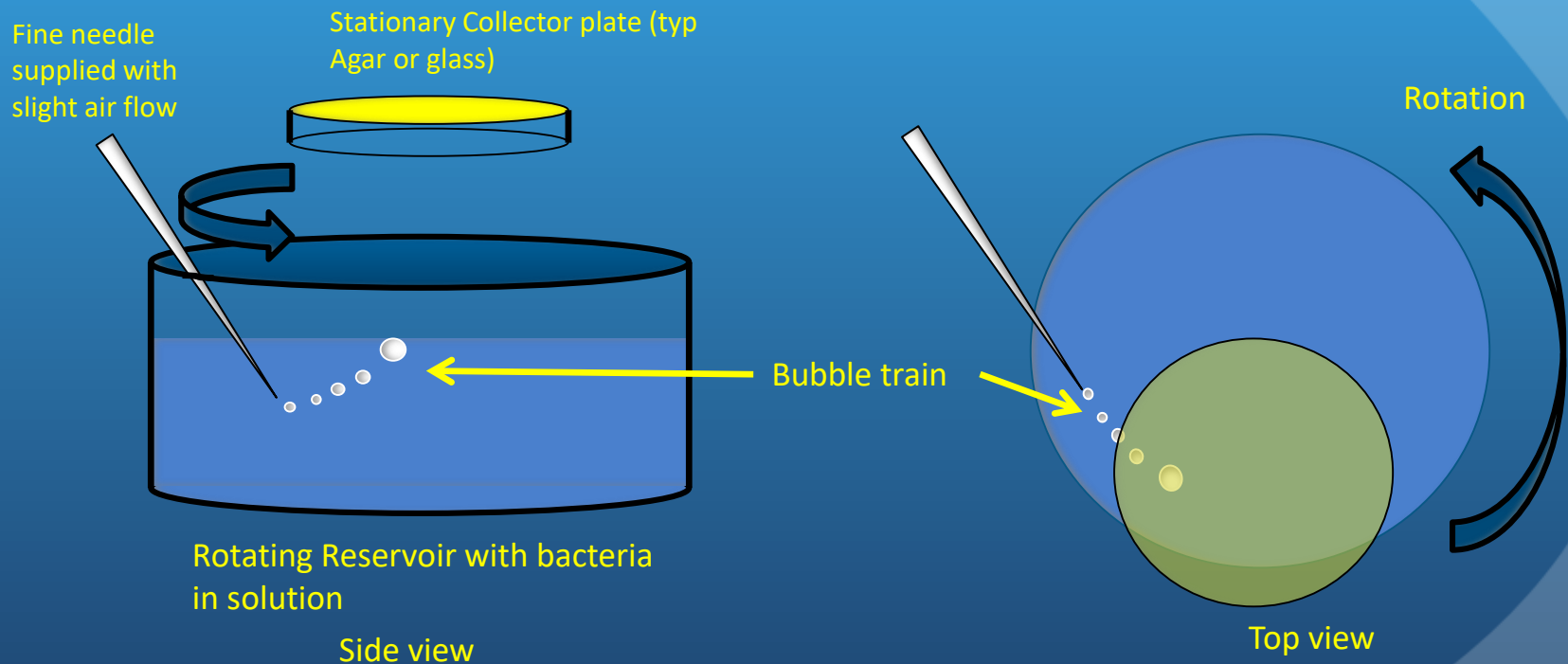
On Earth, “bubble scrubbing” is a key player in microorganism dispersal



- Bacteria concentrate in jet drops and film drops
- This plays an important role in a variety of Earth processes, including the spread of disease
- The concentration is species-dependent and depends on bubble size and transit distance through the fluid

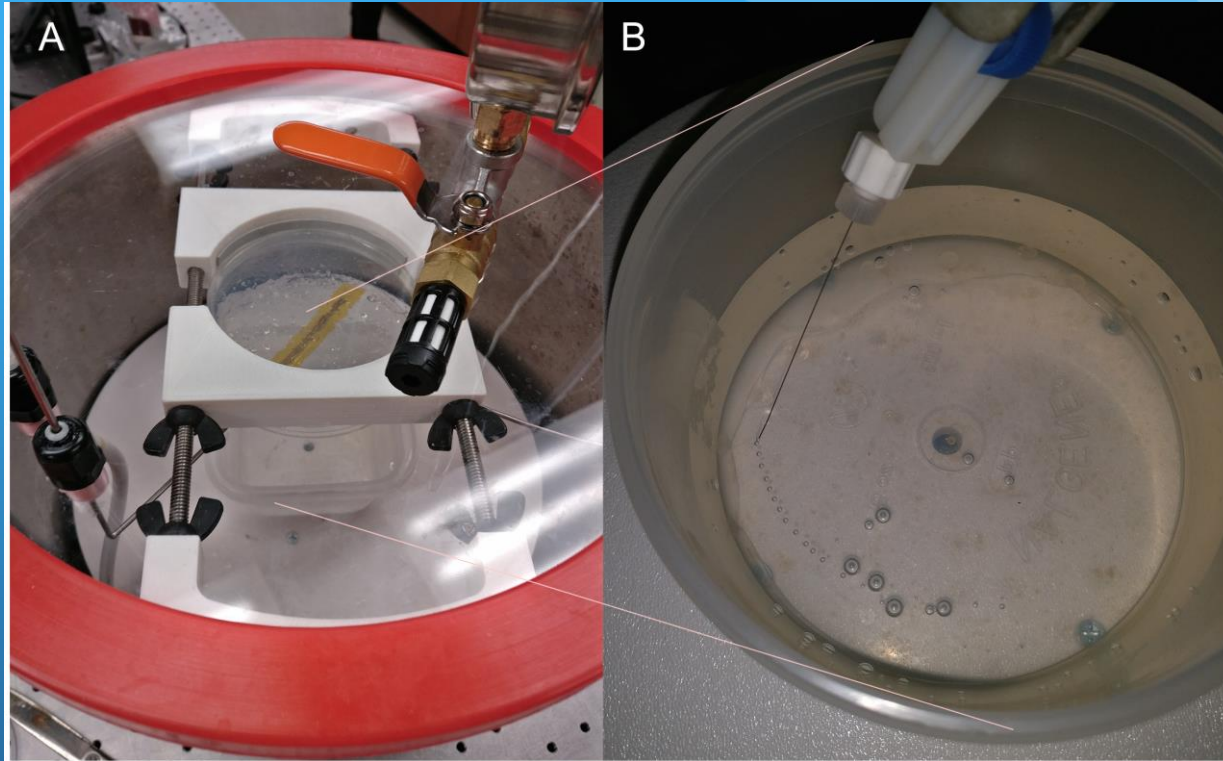
Experiments: Measure concentration factors

Jet drop generator lets us produce controlled bubbles and droplets



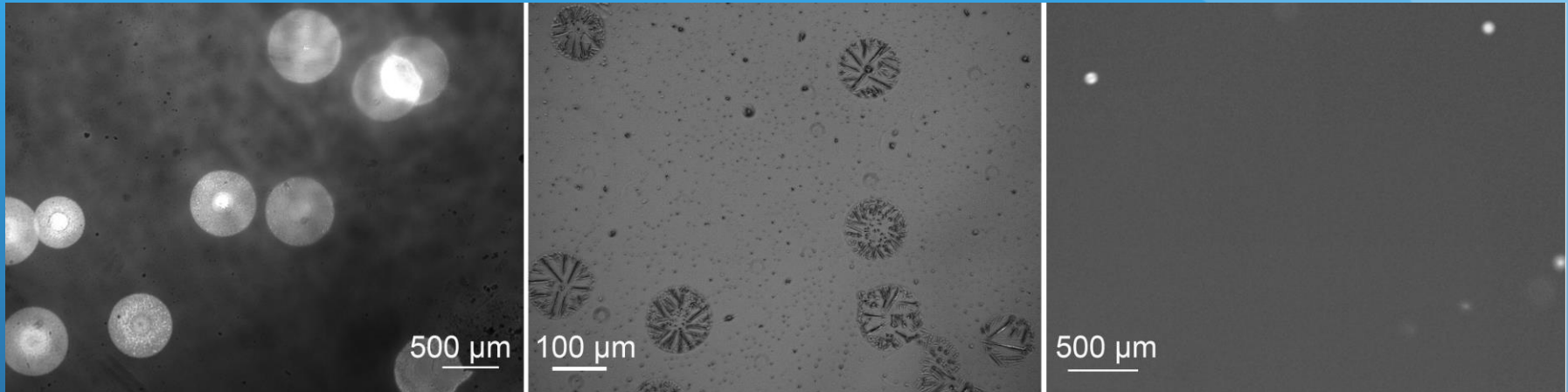
Reservoir rotates so that each bubble rises through undepleted solution

Simple controlled experiment



- Drops can be made to go through undisturbed fluid by using a turntable. Do in atmosphere or under vacuum.
- Number captured can be small. Use culture plates to count bacteria. Visually count number of drops that hit the plate and calculate volume by measuring drop size (as shown on next)
- Demonstrate that we can create jet drops and catch them in media, check that bacteria survive droplet formation

Results



Vacuum drops
(captured on soot
covered cover slip)

Atmosphere drops
(captured on cover
slip)

Shadowgraph
(in atmosphere)

Drops in vacuum are larger than under atmosphere. This affects concentration factors. Otherwise, presence of vacuum does not seem to affect bacterial concentration, and has small effect on survival

Concentration factors in droplets vs. reservoir

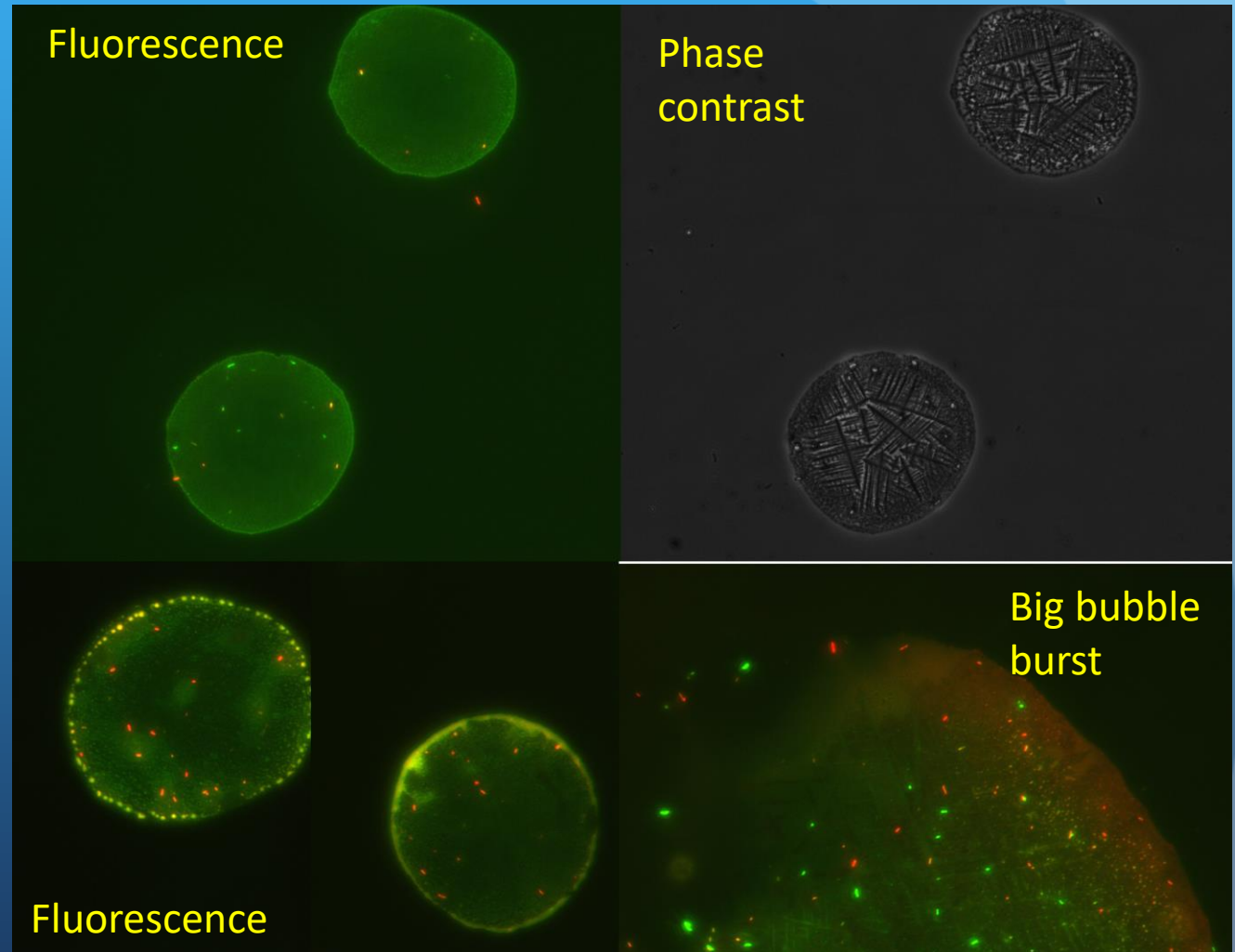
- *Bacillus subtilis*: ~1
- *E. coli*: ~1
- *Serratia marcescens*: ~5000-10000
- Calculate as # cells/mL by serial dilution of reservoir compared with known number & volume of droplets
- At least 4 plates for each condition



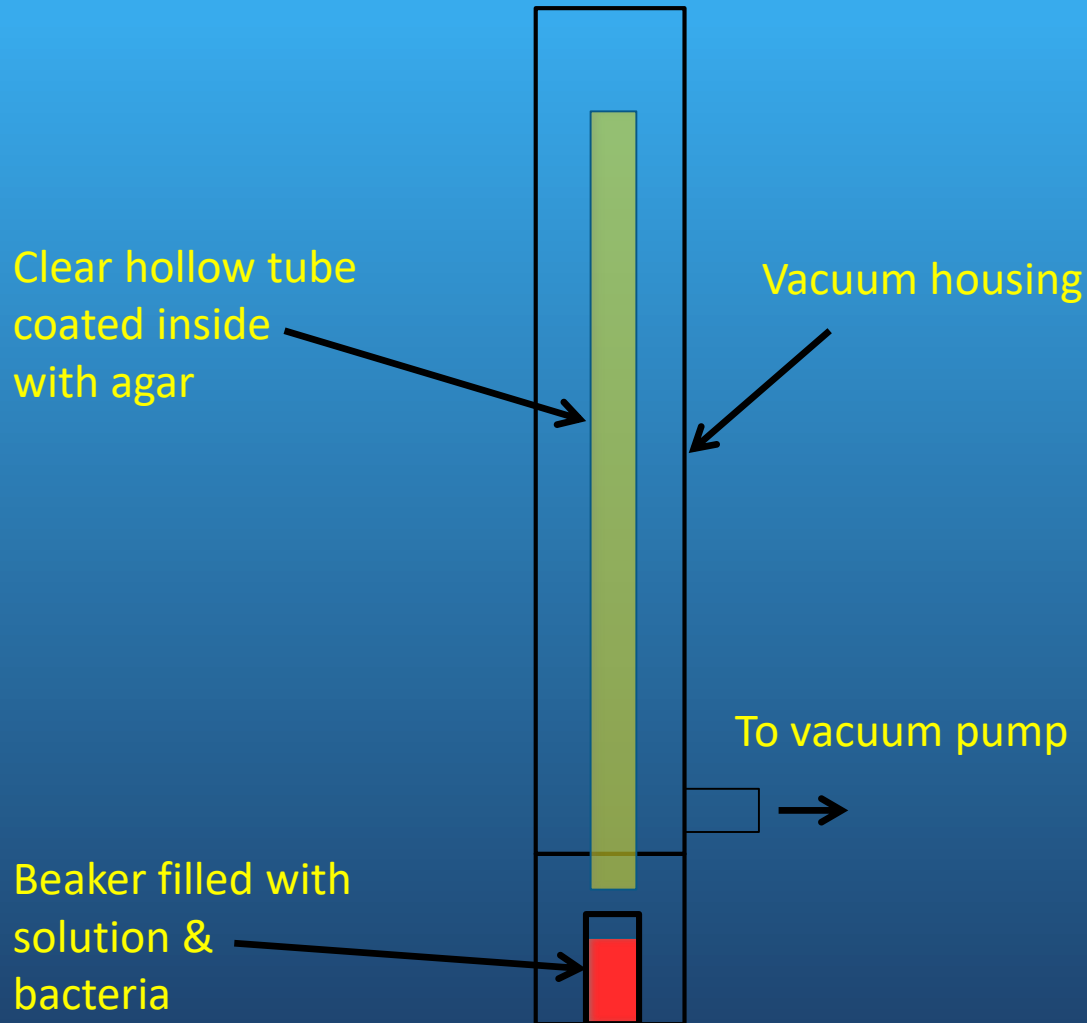
Manual counting of live vs. dead cells in droplets: more cells die under vacuum

Green = Live
Red = Dead

Atmosphere →



Experiments: Escape of droplets and bacteria from the bulk fluid



Whole assembly sits inside -14 C cold room.



Boiling and exsolution into a tall column



Gentle Boiling (L) Carbonated (R)

- Performed in -14 °C cold room
 - Column and Agar precooled to -14 C
 - Reservoir cooled to triple point
- 2 m tubes coated with LB-agar placed above reservoir of 0.9% saline with $\sim 10^5$ cells/mL *S. marcescens*
- The whole thing placed inside vacuum
- First tube: reservoir degassed; the only bubbling is triple point boiling
- Second tube: carbonated reservoir; bubbling is exsolution of CO₂ as well as boiling
- Both exposed for 10 min then incubated for 48 hours to see growth of the bacteria
- Extensive growth seen in both models - many colonies in both tubes
- Can't easily measure concentration or dilution because droplets aren't controlled

Summary & Conclusion

- We've built up systems to test bacterial survival in the transition from bulk fluid to the beginnings of plumes for two models
- Bacteria can survive the transition from bulk to droplets in both.
- The drop formation process does capture bacteria, and can also concentrate relative to the bulk fluid
 - Concentration factor depends on bacterial strain
- Further tests planned:
 - Initial velocity measurement of droplets in plume simulator
 - High velocity capture survivability of frozen bacteria in droplets
 - Dilution/concentration of various bacterial strains in drop formation